EFFECTS OF PHOSPHORUS-32 ON THE COTTON RAT

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One of the useful applications of radioisotopes in ecology is in tagging animals so that their movements can be traced with portable radiation detectors. Griffin (1952) and Pendleton (1956) have discussed various aspects of this technique.

Among vertebrates, radioactive marking has thus far been used most extensively in studies of mammals. Several types of rings or bands utilizing Co^{60}, Ag^{110}, I^{131}, and Sb^{124} as radiation sources have been used for tagging moles, shrews, bats, and mice (Godfrey, 1953, 1954a, 1954b, 1955; Gifford and Griffin, 1960; Linn and Shillito, 1960; Punt and van Nieuwenhoven, 1957). Kaye (1960, 1961) and Johanningsmeier and Goodnight (1962) marked mice with internal tags employing Au^{198} and I^{131} as radiation sources. In addition to their use as inert tags, radioisotopes have been employed in a manner that results in their incorporation into the metabolic systems of the marked individual. Jenkins (1954) marked a lemming (*Lemmus*) with radioactive phosphorus given in the food. Miller (1957) studied the home range of a meadow mouse (*Microtus pennsylvanicus*) by injecting P^{32} into the abdominal cavity and then tracing the movements of the animal through radioactive excretory products deposited on dropping boards. Similarly, Birkenholz (1962) obtained data on movements of round-tailed muskrats (*Neofiber alleni*) by scanning feeding pads with a geiger counter for the presence of radioactive feces from individuals previously live-trapped and injected with P^{32}.

In any system of marking animals for field studies, the possible effect of the marking method on the activities of the animals must be taken into consideration. Although workers who have marked wild mammals with radioisotopes have given consideration to the type and strength of radiation necessary to detect tagged individuals under the conditions of the study, relatively little attention has been given to the possible effects of radiation from the tag source on the behavior of the marked animals.

Our objectives in the present study were (1) to test the relative effectiveness of different doses of P^{32} in labeling the feces of the cotton rat (*Sigmodon hispidus*) as a preliminary to field studies and (2) to obtain some indication of the influence, if any, of the radiation levels on the animals. The criteria selected for evaluating radiation effects were general behavior and appearance, trends in body weight, and patterns of food and water consumption.

Materials and Methods

Twenty-four cotton rats live-trapped in the vicinity of Gainesville, Alachua County, Florida, were used. The group consisted of 12 males and 12 females, ranging in weight from 71 to 166 g (mean, 119.2 g). The animals were individually housed in metal cages equipped with wire mesh bottoms and pans. Food (Purina rat chow) and water were provided *ad libitum.*

After a minimum acclimation period of two weeks in the laboratory, the animals were divided into four groups of six individuals each. Each group had an approximately equal weight distribution and sex ratio. Three groups were given intraperitoneal injections of different amounts of P^{32} in unbuffered solution; the fourth group served as controls. The treated rats received doses of isotope equivalent to 10 (Group I), 2 (Group II) and 0.5 (Group III) $\mu c/g$ of body weight. The controls (Group IV) received injections of distilled water on the same fluid volume/body weight basis as the treated individuals.

The radioactivity of feces of treated animals was measured daily. A sample of ten fresh pellets was randomly selected from the pan beneath the cage of each animal, and the activity (counts/minute) of each pellet was determined separately with a Geiger counter. The tube face was positioned one inch from the pellet when a reading was made.

Observations were made each day on the general appearance and behavior of the animals during a 14-day period before and 30-day period after treatment. Food and water consumption were measured daily in all groups for two weeks prior to treatment and for 30 days after treatment. The amount of food consumed each day was calculated by subtracting the weight of uneaten food from the known weight given the previous day. Water intake was measured by means of calibrated water bottles fitted with drinking tubes. A bottle and tube in an empty cage were used to correct for evaporative losses.

Weights of all animals were recorded at the beginning of the experiment. Rats dying during the course of the study were weighed and autopsied. At the end of the 30-day interval following treatment, the surviving animals were sacrificed, weighed, and autopsied. Weights of adrenal glands and spleens were obtained after preservation in 10 percent formalin. The organs were blotted to remove excess moisture and weighed on an analytical balance to 0.1 mg.

RESULTS

P^{32} elimination in feces: Figure 1 shows the relationship between dosage of P^{32} and the level of radioactivity of feces at 9, 18, and 24 hours post injection and thereafter at daily intervals through 21 days. The points plotted are averages of the highest counts in the fecal samples of each animal in the group and thus represent maximum activity.

The data on P^{32} elimination indicate that the level of activity of the feces reflects the strength of the dose given. However, the ranges of activity in the three groups overlapped broadly, and considerable variation in the activity of pellets occurred within and between samples from individuals of the same group. The extent of this variation was correlated with dosage, being most pronounced in Group I, intermediate in Group II, and least in Group III.

The level of activity of at least some of the fecal pellets of each animal in all groups was still sufficiently above the background level to be detectable at the end of 21 days. The carcasses of animals in all groups were still highly radioactive and could be easily recorded at a distance of several feet with a Geiger counter at the end of 30 days.

General health: Mortality occurred only in Group I. All but one of the animals in this group died within the 30-day period following injection. Mean survival time was 12.8 days, with individuals dying from 5 to 21 days after injection. The lone survivor appeared to be normal in all respects when sacrificed at the close of the study. The animals that died showed no obvious effects of radiation until shortly before death, when they began to huddle in a corner of the cage with the pelage ruffled. The cotton rats in Groups II and III exhibited no outward changes in appearance or behavior as the result of treatment.

On the basis of gross examination at autopsy, only individuals in Group I showed signs that could be attributed to possible radiation damage. One specimen had a small cyst about ¼ in. in di-

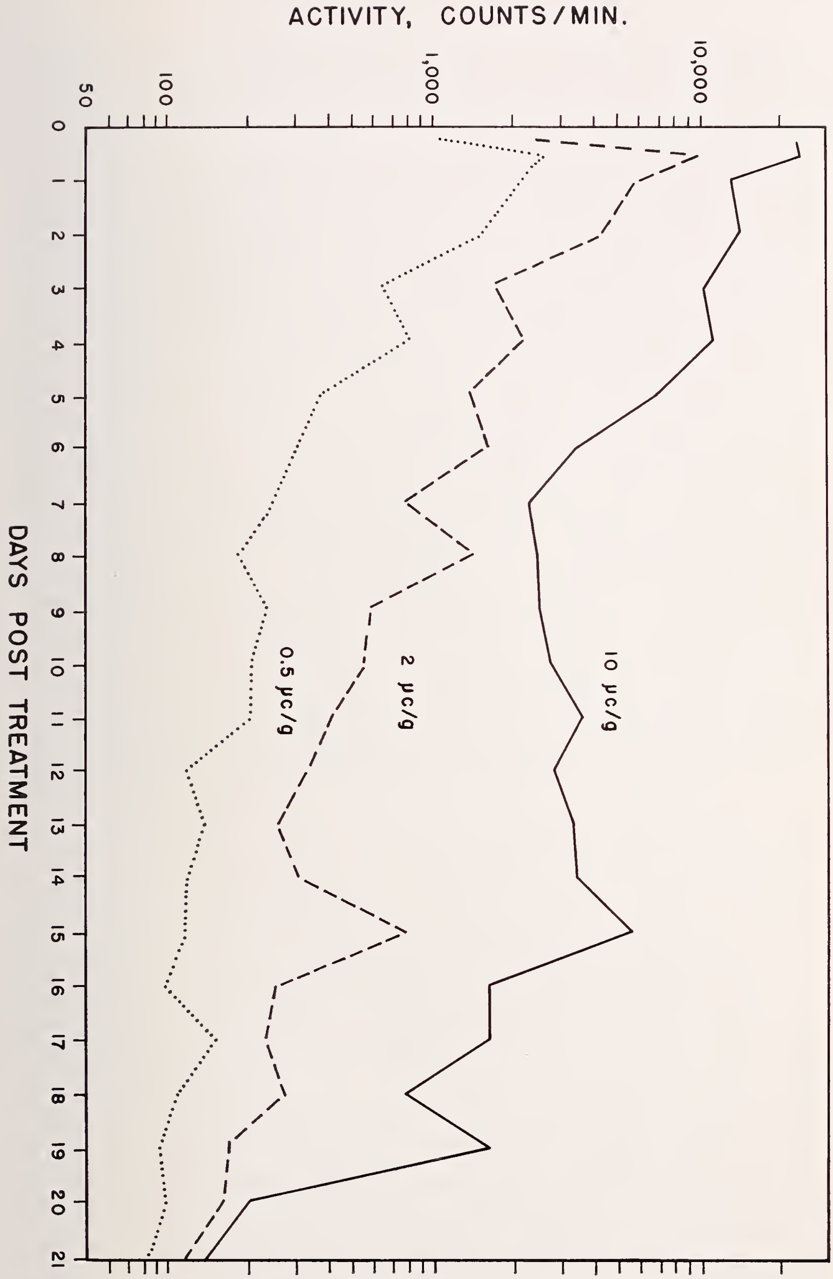

Fig. 1. Radioactivity over a 3-week period of fecal pellets of cotton rats receiving intraperitoneal injections of P^{32} equivalent to 10, 2, and 0.5 $\mu c/g$ of body weight.

ameter on the caecum. Another had a large ulcerated area on the stomach, and its prostate glands and seminal vesicles were distinctly abnormal in appearance. A third animal had conspicuous lesions on the liver.

A reduction in spleen weight appeared to be correlated with the amount of isotope received. Mean relative weights of spleens, expressed as mg of spleen/g of body weight, were: Group I, 1.469; Group II, 1.583; Group III, 1.614; and Group IV, 1.658. Mean relative weights of adrenal glands were: Group I, 0.368; Group II, 0.366; Group III, 0.463; and Group IV, 0.387.

Body weight: Differences in trends of body weight between treated and control groups were apparent. Every control animal except one gained weight during the course of the experiment, the animals averaging 17 percent heavier at the end of the 30-day period following treatment. In treated groups, the extent of weight change was correlated with dosage. Only the surviving animal in Group I gained weight during the test. When sacrificed it weighed 7 percent more than its initial weight. The remaining individuals in this group had lost an average of 13 percent of their initial weight by the time of death. The mean loss of weight for the group as a whole was 9 percent. The rats in Groups II and III gained weight, but to a lesser extent than controls, averaging 10 and 11 percent heavier, respectively, at the end of the experiment.

Food consumption: The mean daily food consumption of the six control individuals over a 42-day period of measurement was 15.8 g, or 0.14 g/g body weight.

To provide a basis for a comparison of the effect of dosage on food consumption patterns, the mean daily food intake for three-day periods was calculated for each group over the 30-day post treatment interval and then expressed as a percentage of the mean consumption per day for that group during the six-day period immediately preceding injection. All treated groups took less food after injection than before (Figure 2). In contrast, the food intake of controls increased markedly during the same period. The cotton rats receiving the heaviest dose of isotope exhibited the greatest reduction in food consumption. Although the differences were not pronounced, the rats in Group II tended to consume less food than those in Group III. All the Group I fatalities drastically reduced their food intake or stopped feeding entirely from one to five days prior to death.

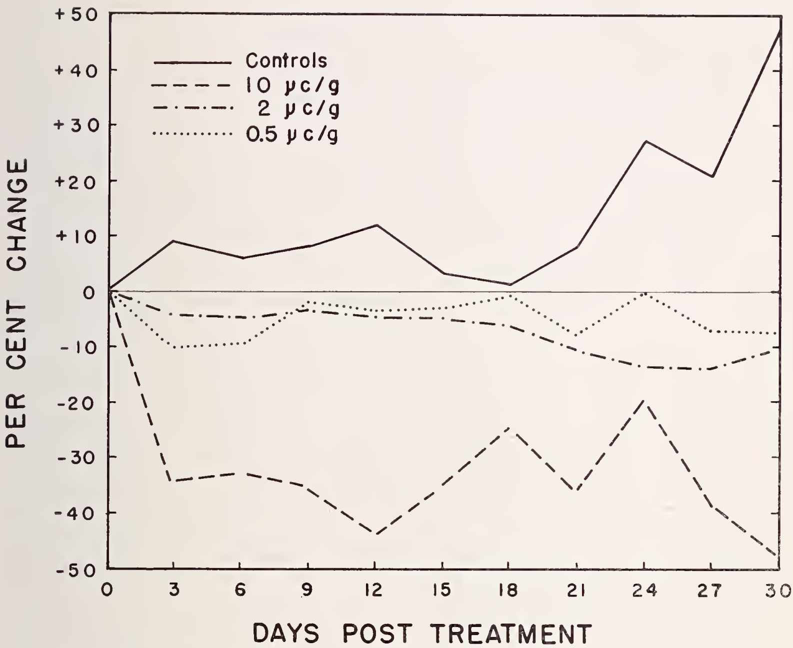

Fig. 2. Effect of different doses of P^{32} on food consumption of cotton rats. Average daily food consumption over 3-day periods is plotted as a percentage of mean daily food intake for the 6-day interval immediately preceding treatment.

Water intake: Over an interval of 42 days, control animals drank an average of 24.4 ml of water per day, or 0.22 ml/g body weight.

Data on water consumption presented in Figure 3 are expressed in the same way as those for food. The trends in water consumption of Group I following treatment agree generally with those for food, with the exception that the decline in drinking occurred more gradually than that of feeding. Although the food intake of Groups II and III declined following injection, their water consumption tended to increase. It is not known whether the difference in water intake between these groups from about 6 to 15 days after treatment is attributable to the different levels of radiation involved. Except for an initial depression following injection with water, the trend of fluid intake of controls conformed closely to that of food consumption. It appears, therefore, that treated individuals had higher water requirements relative to food intake than controls.

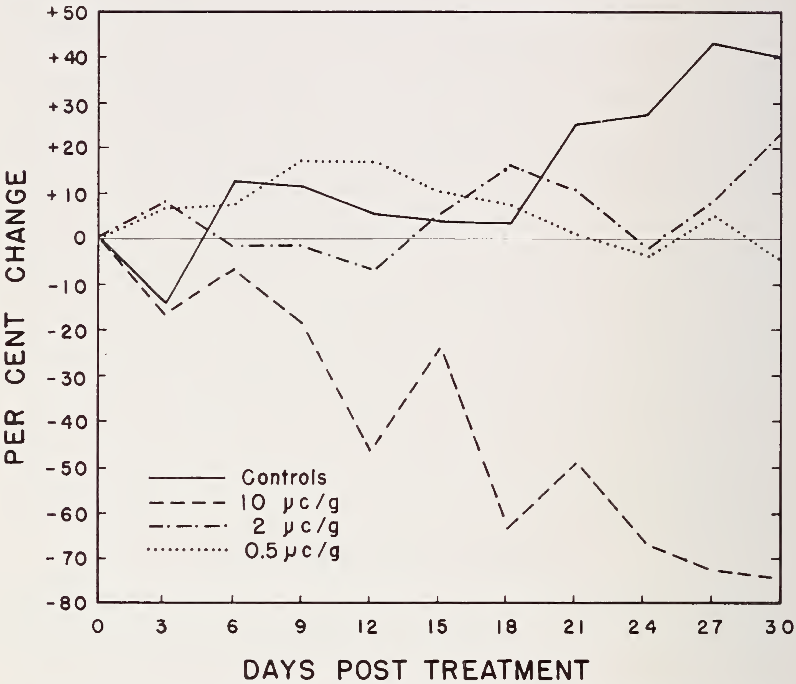

Fig. 3. Effect of different doses of P^{32} on water consumption of cotton rats. The data are expressed in the same way as those for food consumption in Figure 2.

Daily variation in water consumption in all groups was greater than that of food consumption. Group I rats that died drank little or nothing from one to seven days preceding death.

Discussion

The effects of radiation on the physiology and behavior of a mammal marked with radioisotopes could conceivably influence a number of ecologically significant aspects of its biology, as, for example, activity cycles, food and water requirements, pattern of movements, exposure to predation, susceptibility to parasites and disease, reproduction, and social interactions. As yet, however, workers who have used radioisotope marking techniques in studies of wild mammals have given little consideration to the extent to which the data obtained on tagged individuals may have been biased by such effects. Godfrey (1954a, 1954b), Punt and van Nieuwenhoven (1957), and Gifford and Griffin (1960) have reported that they were unable to observe any harmful effects of radiation on tagged individuals in their studies. In these instances, however, the marked animals were apparently not closely studied for radiation effects; and it is possible that the level of radiation involved, though not high enough to produce obvious effects, was still sufficient to cause behavioral or physiological disturbances that might have biased data obtained from the marked individuals.

The LD_{50}/ 30-day dose of P^{32} for the cotton rat is approximately 4.3 μc/g body weight (Hankins, 1954). This value is similar to that, about 4.5 μc/g, for the laboratory mouse and rat (Koletsky and Christie, 1951; Mewissen and Comar, 1959). The heaviest dose used in this study was, therefore, over twice the LD_{50} level, but the other two were only 0.4 and 0.1 of this value. Based on the changes in weight and patterns of food and water consumption following treatment, each of these doses had some influence on the animals, and the response of weight and food and water intake are in turn probably symptomatic of a variety of physiological and behavioral changes. Although only rats in Group I showed gross behavioral changes, and then only when close to death, we strongly suspect that with more critical observations and testing behavioral changes would have been demonstrated in the animals receiving even the lowest dose. The magnitude of the effects of radiation on weight, feeding, and drinking in Group I suggest that field data on home range or other parameters derived from animals marked with a similar dose would not be representative of the unmarked population. Whether or not the effects of the lower dosages would produce significant biases under field conditions is problematical, but the possibility cannot be ignored. Even the relatively slight modification of food and water intake occurring in the animals of Group III might reflect somewhat altered activity patterns which under field conditions might influence the animal's movements or other activities.

In view of the foregoing, it is possible that doses of P^{32} that have previously been used in actual field studies may have been too high to give reliable results. Jenkins (1954) marked a lemming with 250 μc. Assuming an average body weight of about 50 g in this mammal, the dosage was equal to approximately 5 $\mu c/g$ body weight. Miller (1957) used nearly the same dose (200 μc with a 41 g animal) in studying the home range of a single meadow mouse. She suggested that an even higher dose, perhaps 400 μc, would have given better results. Assuming that the LD_{50} of P^{32} for these rodents is close to that for *Sigmodon* and the laboratory mouse and rat, it seems doubtful that individuals marked with a dose approximating the LD_{50} would behave normally. This criticism can be extended to at least certain studies in which other marking procedures or types of radiation have been used. For example, Kaye (1960, 1961) used gamma emitting tags with a strength of up to 4500 μc to trace the movements of harvest mice.

There is clearly a need for further studies on the effects of different radioisotope marking methods on wild mammals before ecological information obtained with this technique can be fully evaluated. In addition to laboratory investigations designed to obtain more detailed information on the less conspicuous effects of lower doses of radiation on behavior or aspects of physiology that may have particular ecological significance, it would seem desirable to test the effects of various types of radioactive marking under natural conditions. This could perhaps be done by marking some animals in a given population with the types or strengths of radioactive tags to be tested and others by conventional methods to serve as controls. The population could then be studied by live-trapping procedures and the relationship of type of mark used to trap response, home range size, longevity, parasite loads, weight, or other aspects of life history or ecology determined.

SUMMARY

Three groups of cotton rats (*Sigmodon hispidus*) were given intraperitoneal injections of P^{32} equal to 10, 2, and 0.5 $\mu c/$ g of body weight in order to obtain data on the rate of isotope elimination in feces and the effects of the different levels of radiation involved on behavior and appearance, weight, and food and water consumption patterns. A fourth group served as controls. Elimination of the isotope in the feces was roughly proportional to dosage. Mortality occurred only in the 10 $\mu c/g$ group, but trends in body weights of all treated groups differed from those of controls. Rats receiving the heaviest dose exhibited a pronounced

reduction in food consumption following injection. The food intake of the intermediate and low dosage groups declined to a lesser extent, while that of controls increased markedly. Water consumption in cotton rats receiving 10 $\mu c/g$ body wt. was markedly reduced following treatment but declined more gradually than food intake. Animals given 2 and 0.5 μc doses exhibited a tendency toward greater water consumption after injection than before, despite a reduction in the amount of food taken. It is concluded that the changes in weight and feeding and drinking patterns following administration of P^{32} observed in this study were probably symptomatic of physiological and behavioral effects that under field conditions might have a significant influence on activity, home range, or other aspects of life history or ecology being studied.

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